Transfer of antibiotic resistance in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a serious human pathogen with remarkable adaptive powers. Antibiotic resistant clones rapidly emerge mainly by acquisition of antibiotic resistance genes from other *S. aureus* strains or even genera. Transfer is mediated by a diverse complement of mobile genetic elements and occurs primarily by conjugation or bacteriophage transduction with the latter traditionally being perceived as the primary route of transfer. Recent work on conjugation and transduction suggest that transfer by these mechanisms may be more extensive than previously thought both in terms of the range of plasmids that can be transferred by conjugation and the efficiency with which transduction occurs. Here, we review the main routes of antibiotic resistance gene transfer in *S. aureus* in the context of its biology as a human commensal and a life-threatening pathogen.

STAPHYLOCOCCUS AUREUS AND MOBILE GENETIC ELEMENTS

The development of antibiotic resistances is a key threat to humanity. The World Health Organisation recently categorised the global resistance threats and among the organisms of high priority is *Staphylococcus aureus*. *S. aureus* is a serious opportunistic pathogen that colonizes a third of the healthy human population and yet is the leading cause of bacterial infections in the developed world, giving rise to a variety of conditions ranging from benign skin infections to fatal infective endocarditis and necrotizing pneumonia. Treatment of staphylococcal infections has traditionally been with β-lactams but as methicillin resistant *S. aureus* (MRSA) strains have spread globally alternative treatment involves vancomycin, daptomycin and linezolid. While acquired resistance to vancomycin encoded by the *vanA* gene is rarely observed vancomycin intermediate *S. aureus* (VISA) is a growing concern as vancomycin treatment failure is prevalent in infections with such strains (1). MRSA treatment is further complicated by the emergence of strains that are resistant to the oxazolidinone drug, linezolide through mutations in the 23S rRNA and the ribosomal methyltransferase encoding *cfr* gene (2) as well as strains showing reduced susceptibility to the lipopeptide, daptomycin (3).

The core genome of *S. aureus* strains is highly clonal and is divided into lineages defined by clonal complexes (CC) of sequence types (STs). Strains are also grouped based on epidemiology in hospital-, community- or livestock-associated (HA, CA and LA, respectively). In contrast to the core genome the diversity of mobile genetic elements in *S. aureus* is remarkably variable. In fact, 15-20% of the genome is composed of mobile genetic elements including bacteriophages (phages), pathogenicity islands, plasmids, transposons, integrative conjugative elements (ICEs), integrons and staphylococcal chromosome cassettes (SCC) where all elements except the phages may carry antibiotic resistance genes (ARGs) (4, 5, 6).

Most clinical isolates of *S. aureus* contain plasmids in the size range of 1 to 60 kbp. They commonly carry from one to multiple ARGs with the small plasmids typically providing resistance to erythromycin, chloramphenicol or tetracycline while the larger plasmids encode multiple ARGs providing resistance to β -lactams, aminoglycosides and macrolides (7). In the larger conjugative plasmids ARGs are often integrated as part of mobile genetic elements such as transposons that can provide resistance to aminoglycosides, erythromycin,

spectinomycin, tetracycline, trimethoprim, vancomycin or β-lactams (Table 1, supplementary information).

Conjugative transposons or integrative conjugative elements (ICEs) are a diverse group of mobile genetic elements that reside in the host chromosome but retain the ability to integrate into and excise from bacterial chromosomes and transfer themselves by conjugation. Two distinct families of ICEs, represented by Tn*5801* (8) and ICE*6013* (9) have recently been identified in *S. aureus*. Tn*5801* belongs to the Tn*916* family, which is a prototypical conjugative transposon that encodes tetracycline resistance and is broadly distributed among many different Gram-positive bacteria (10). ICE*6013* is widespread in *S. aureus*. Remarkably, ICE*6013* can also carry Tn*552*, which encodes penicillin resistance (9).

Staphylococcal cassette chromosomes (SCCs) are relatively large MGEs (20-55 kbp) inserted into the *S. aureus rlmH* gene. The methicillin resistance gene *mecA* is common in SCCs in which case they are named SCC*mec* and confer resistance to β-lactam antibiotics in the MRSA strains (see box 2). Resistance can also be provided by integrated transposons or plasmids as for example the Tn*554* transposon carrying *ermA* and *aad9* genes encoding resistance to erythromycin and streptomycin/spectinomycin (11). Although widely present in different staphylococcal species, the mechanism by which the SCC*mec* elements are transferred remains unsolved. Interestingly, the SCC*mec* elements encode an active MCM-like helicase, suggesting these elements may be replicative (12). However, the exact role of this protein in the SCC*mec* cycle remains to be determined.

While bacteriophages rarely carry antibiotic resistance genes themselves they appear central in the mobility of ARGs in *S. aureus*. However, the related *S. aureus* pathogenicity islands, SaPIs, which employ the machinery of the phages for replication and dissemination, but otherwise reside integrated in the bacterial chromosome (13) have been reported to encode ARGs (14). Thus, *S. aureus* carries a plethora of MGEs that either alone or in combinations offer a plentiful source of antibiotic resistance. Despite the antibiotic resistance challenges we know surprisingly little of how ARGs are transferred between strains, species and even genera and even less of how environments and gene expression levels influence transmission. Here we will focus on recent advances in our knowledge of how ARGs are transferred in *S. aureus*, the environments where it may be happening and the barriers (or lack of such) that limit transfer.

MECHANISMS OF GENE TRANSFER

Conjugation

Conjugative transfer of plasmid encoded resistance genes has long been recongnized as a key mechanism by which antibiotic resistance genes disseminate. Instrumental in this process are conjugative plasmids that carry clusters of genes that encode products required for cognate plasmid transfer and the mobilization of plasmids that encode limited conjugation genes or none at all (Figure 1). The plasmid encoded relaxase (Nes) recognizes, cleaves and attaches to the origin of transfer, *oriT*, located on the plasmid and assisted by accessory proteins in a relaxome complex, the DNA is transferred to the recipient cell through a type-IV secretion system (Figure 1) (16, 17). Conjugation is also the mechanism used by ICEs to promote their transfer. Although the *S. aureus* ICEs have not been extensively characterised, all of them encode the genetic functions required for conjugation such as excision and circularization of the ICE in addition to coupling protein and mating channel (18).

One of the most well-characterized conjugative plasmid families in *S. aureus* comprise pSK41 (19). They commonly provide aminoglycoside resistance (20, 21) but may also confer resistance to a variety of other antibiotics including penicillin, mupirocin, tetracycline, macrolides and vancomycin (22, 23, 24, 25). pSK41 is a 46.4 kb plasmid that in addition to genes encoding replication, stability and conjugation functions contains a number of transposon-like structures and co-integrated plasmids flanked by copies of the insertion sequence IS*257*, which itself contains resistance elements (24). Importantly, the antibiotic resistance genes appear to be introduced into the plasmid by these co-integrated elements (24) and the ability of IS*257* to capture small resistance plasmids may be a key factor in the formation of resistance gene clusters (26). Mobility of the SCC*mec* cassette has been difficult to reproduce in laboratory settings but one way has been to capture the cassette on a pSK41/pGO1 plasmid following overexpression of the SCC*mec* recombinase and IS*257/431* recombination demonstrating the power of IS elements in recruiting antibiotic resistance genes to conjugative plasmids (27).

Until recently conjugation was considered a relatively rare event in *S. aureus* as only about 5% of the plasmids encode all the products that are required for autonomous transfer (15). However, we now know that conjugative plasmids promote transfer of other plasmids

(Figure 1). The mobilizable plasmids carry an *oriT* and encode a corresponding relaxase but lack the genes required for mating pore formation and coupling protein. For transfer, the relaxases of these plasmids must therefore recognize the coupling protein of the conjugative plasmid (28, 29). An *oriT* sequence alone may even be enough for transfer as it was recently was demonstrated that the conjugative plasmid, pWBG749 can transfer plasmids that carry oriT-mimicking sequences presumably being recongnized by the pWBG749 relaxase (30). Importantly, sequences resembling the *oriT* of pWBG749 are present on half of all sequenced *S. aureus* plasmids including the large plasmids, pIB485, pMW2 and the USA300 p03 family indicating that this type of transfer may be frequent in *S. aureus* (30, 31). Even in the absence of any transfer related loci transfer may occur for plasmids that replicates by rolling-circle replication (Figure 1). In *B. subtilis* is was demonstrated that the replicative relaxase (*rep*) required for rolling-circle replication acts as a mobilization relaxase that upon recognition of the single strand replication origin, oriV nicks DNA and initiates plasmid DNA transfer (32). With these new findings it appears that most if not all plasmids in *S. aureus* are in fact transferrable in the presence of conjugative plasmids or perhaps just ICE elements and that the distribution of elements encoding the basic transfer machinery including the mating pore and coupling protein should be studied in greater detail.

Bacteriophage transduction

In addition to plasmids, *S. aureus* strains commonly harbor one to four functional bacteriophages integrated in their genome as prophages and they have long be recognized as being involved in evolution of *S. aureus* strains and for human colonization (33,34, 35, 36, 37). Prophages can excise either spontaneously or by induction via activation of the bacterial SOS response that is triggered in response to DNA damage elicited by for example oxidative stress or exposure to some antibiotics (38). After induction the prophage enters the lytic cycle leading to production of phage progeny and lysis of the host cell. During the lytic cycle, bacterial DNA instead of phage DNA may be packaged into the phage capsid producing a transducing particle, which upon release from the (donor) host cell can transfer this bacterial DNA to another (recipient) cell – a phenomenon known as generalized transduction (Figure 2a) (39). While transduction previously was thought to be a result of

errors in the phage packaging machinery, recent data suggest this is not the case as the ratio of transducing particles to virulent phage varies upon prophage induction by different antibiotics (41). Generalized transduction relies on the phage machinery recognizing pseudo *pac*-sites that mimic the phage pac-site from where phage DNA packaging is initiated during lytic growth. During transduction 45 kbp of bacterial DNA (approximately 1.5% of the *S. aureus* genome) is packaged into the transducing particles and both chromosomal and plasmid DNA can be transferred (40, 42). In *S. aureus,* generalized transduction was previously thought to be restricted to temperate phages with those belonging to serotypes B and F being mostly studied (43). In molecular biology the serotype B phages ϕ11 and 80α have been used extensively as tools for transfer of mutations and genes between strains.

In addition to plasmids, other moble genetic elements may also be transferred by generalized transduction including the SCC*mec* cassettes. The SCC*mec* DNA (44) or fragments hereof (45) can be packaged into transducing particles, but transduction is inefficient. A prerequisite for transduction of SCC*mec* is the presence of a penicillinase plasmid in the recipient strain possibly due to transcriptional regulation of the *mec* gene by the plasmidborne *blaR1-blaI* regulatory genes and transduction is occasionally associated with deletions in the SCC*mec* (46). Further, the size of the SCC*mec* (20 kbp to 60 kbp) is limiting the transduction efficiency of the element owing to capacity limitations of the phage capsid. Thus, only the smaller SCC*mec*s are expected to transfer through generalized transduction as demonstrated for SCC*mec* type IV and SCC*mec* type I (46). However, as also generalized transduction of the penicillinase plasmid has been demonstrated (47), it is likely that generalized transduction is a route of transfer for at least some SCC*mec* elements between *S. aureus* strains.

More recently transduction has been reported for phages not belonging to serotypes B and F (44, 48, 49). One prominent example is Φ187 among *S. aureus* strains that only binds and transduces ST395 strains, which harbor an unusual teichoic acid in the cell wall, but surprisingly also other staphylococcal species than *S. aureus* and *Listeria monocytogenes* where a similar structure is found (50). Further, transduction requires adsorption and DNA injection but not the lytic action of the transducing phages (51). These examples suggest that in nature, generalized transduction may be more common than previously anticipated as also reported for other bacterial genera (52). Thus, in addition to being a highly useful

genetic tool, generalized transduction is also a major driver of *S. aureus* evolution and spread of ARGs.

Recently, we discovered a variant of generalized transduction termed autotransduction as an effient way for staphylococcal lysogens to acquire DNA from phage susceptible cells. In autotransduction, phages spontaneously released from a subpopulation of lysogenic cells can infect, lyse and efficiently transfer DNA from a phage susceptible bacterial population back to the intact lysogenic population, which itself survives because of immunity to phage killing (53) (Figure 2b). Driven by spontaneous release of phages from the lysogen this mechanism allows lysogens to acquire chromosomal DNA and different MGEs, including plasmids or SaPI elements, so effectively that during antibiotic selection the lysogens survive in competition with an antibiotic resistant but non-lysogenic strain (53).

ARGs may also be spread via the *S. aureus* pathogenicity islands (SaPIs). The dissemination of SaPIs occurs when the SaPIs make use of the machinery and structural proteins of helper phages that are redirected to produce SaPI particles, which are transferred with very high efficiency (13). Importantly, although only a subset of these elements present in different staphylococci encode ARGs, the inter-generic transfer of some SaPIs suggests that they may have a role in the spread of certain ARGs (54, 55). Further, it was recently shown that SaPIs have been involved in a novel variant of generalized transduction, called island-mediated transduction. This mechanism involves coordinated packaging of bacterial DNA into SaPI capsids from pseudo-pac sites in the chromosome, and was shown to transfer large regions of chromosomal DNA with high frequency (56).

Transformation

The uptake of naked DNA by natural transformation requires a series of dedicated competence factors and orthologues of such are naturally encoded by the *S. aureus* genome. It was not until recently, however, it was demonstrated that *S. aureus* is capable of becoming naturally competent. In *S. aureus,* expression of competence genes is regulated by an alternative sigma factor, SigH. Normally SigH is not expressed, but following either a gene duplication or de-repression of post-transcriptional regulation there SigH accumulate to a level that allows natural transformation of *S. aureus* cells with both chromosomal and

plasmid DNA including the SCC*mec*II element (57). More recently cell-wall acting antibiotics have also proven to modulate SigH expression (58). Despite these discoveries there is still no evidence that transformation is a commonly occurring event but it may be that more detailed studies of SigH expression are needed under in vivo conditions in order to identify environments where transformation may be taking place.

TRANSFER DISTANCES AND BARRIERS

In the nasopharyngal cavity *S. aureus* is part of a complex microbiota where it among many other bacteria encounters a wide range of coagulase-negative staphylococci depending on the host colonized (59). All humans are colonized with *S. epidermidis* and transfer between *S. aureus* and *S. epidermidis* has been documented in several reports in the form of conjugative transfer of plasmids encoding resistance to aminoglycosides (20, 60, 61, 62) and linezolid (63). Analysis of more than 300 *S. aureus* and *S. epidermidis* genomes also revealed that SCC*mec* elements appear to be exchanged between *S. epidermidis* and *S. aureus* (64). Although the exact mechanism by which the transfer occurs is largely unknown accumulating data suggest that the cassette originates from *S. epidermidis* which acquired it from other coagulase negative staphylococci (65).

S. aureus also acquires antibiotic resistance from more distantly related bacteria, in particular vancomycin resistance present in enterococci. In 2002 the first MRSA strain was detected that carried *vanA* and displayed a minimum inhibitory concentration (MIC) to vancomycin > 1000 μg/ml. Genome analysis revealed that during co-infection a conjugative *Enterococcus faecalis* plasmid carrying the *van*A gene within the transposon element Tn*1546* had transferred to a MRSA strain. Subsequently the transposons had jumped to a plasmid residing in the original MRSA strain (23). Since then several cases of vancomycin resistant *S. aureus* (VRSA) have been reported, commonly involving the Tn*1546* transposon element either being transferred by an enterococcal inc18-like plasmid or having transferred to a *S. aureus* plasmid (66, 67, 68, 69). The transfer of genetic material from enterococci to *S. aureus* appears to be easier in staphylococcal strains lacking a type III endonuclease and the HsdR restriction modification systems (70) and may be stimulated by

248 the presence of pSK41 family plasmids in recipient *S. aureus* strains possibly in consequence
249 of secreted plasmid factors stimulating transfer (71).

250 Bacteriophages also mediate transfer of DNA between *S. aureus* and other bacterial species
251 (49, 50). Instrumental in this transfer is the presence of unusual wall teichoic acids in some
252 *S. aureus* strains that resembles those of transfer partners and serve as receptors for the
253 transferring phages (50). Particularly, SaPI elements are efficiently transferred by phages
254 between *S. aureus* and the coagulase negative *S. epidermis* or *S. xylosus* (72) and even to
255 more distantly related species such as *Listeria monocytogenes* (54, 55). In the latter cases
256 the authors found that *L. monocytogenes* harbors SaPI-like attachment sites similar to those
257 in *S. aureus* and that transfer could be mediated by several different staphylococcal phages,
258 which were unable to form plaques on *L. monocytogenes*. They concluded that the true
259 overall host range of phages may be much wider if it includes infection without plaque
260 formation, which can be assessed only by gene transfer or phage DNA delivery, a
261 phenomenon recently called silent gene transfer (55, 73).

262 While interspecies transfer does occur the incidents are likely to be rare due to the efficient
263 restriction/modification system that cleaves unmodified DNA upon entry into the cell (74).
264 This system effectively drives the speciation of *S. aureus* in to clonal lineages within which
265 horizontal gene transfer is common but rare between lineages. As the lineages encompass
266 MGEs *S. aureus* seems to have developed an evolutionary compromise allowing genes
267 including MGEs and ARGs to be exchanged very efficiently but usually only between the
268 closely related cells within a lineage (34, 36, 75). Interestingly, however, Roberts *et al.*
269 recently showed that conjugative plasmids carrying ARGs circumvent restriction barriers by
270 losing restriction sites, which are otherwise randomly distributed in *S. aureus* genomes, and
271 consequently can be transferred between lineages (76). Another mechanisms by which
272 bacteria protects themselves against invading DNA is by CRISPR/Cas system (clustered
273 regularly interspaced short palindromic repeats–CRISPR-associated proteins) that is an
274 adaptive immune system against phages and plasmids. Although demonstrated to protect *S.*
275 *epidermidis* against invading plasmids and phages (77) and being present in a broad
276 collection of *S. epidermidis* strains (78), the CRISPR/Cas genes have only been found in a few
277 *S. aureus* strains. Here, however, they have been associated with a SCC*mec* element and
278 shown to provide a barrier to plasmid transformation (79). Despite these limitations, *S.*

aureus has a stunning potential for exchange of genetic material not only between strains but also with other species and even across genera.

WHERE AND WHEN DOES TRANSFER TAKE PLACE?

Transfer of antibiotic resistance has almost exclusively been studied in laboratory settings and therefore, we still know little about where and when transfer occurs in natural settings. One obvious location for transfer to take place is during colonization of a human or animal host. Here, epidemiological studies suggests extensive exchange of mobile genetic elements within lineages of *S. aureus* (37, 80) and in the rare cases of VRSA, the transfer of *van*A and the Tn*1546* from enterococci to *S. aureus* has been shown to occur in co-colonizing strains (67). The efficiency of transfer in "*in vivo*" settings was recently demonstrated experimentally where co-colonization of piglets with *S. aureus* strains carrying various plasmids and bacteriophages revealed that after just 4 hours, transfer of MGEs were observed indicating highly efficient mobility *in vivo* (36).

Another environment where transfer of resistance genes may take place is in biofilms. *S. aureus* forms biofilm during infection and on medical implants (81) and in the absence of surfaces *S. aureus* aggregates potentially involving host proteins (82, 83). During biofilm formation both conjugation and mobilization is strongly enhanced (84). Phage release and thus the potential for transduction is also higher in biofilms compared to planktonic cultures (85) indicating that this environment may be a hot spot for gene transfer. Remarkabily, some MGEs may even promote biofilm formation such as the staphylococcal pathogencity island SaPIbov2 that encodes Bap (86) or the conjugative plasmid pAFS11 carrying genes resembling the *ica* operon responsible for polysaccharide intercellular adhesion (PIA) production (87) suggesting that the MGEs themselves may contribute to formation of favourable transfer conditions.

Generally little is known about the intra- and extra-cellular conditions that affect horizontal gene transfer. During infection, staphylococcal cells are likely to encounter oxidative stress and other DNA damaging components elicited by the innate immune response, which via the SOS response are likely to induce prophages SaPI elements as has been demonstrated for *Escherichia coli* (88) and *Salmonella typhimurium* (89). Sub-lethal doses of antibiotics

such as ciprofloxacin and oxacillin also induce the SOS response that in turn may lead to phage mediated transfer of resistance genes (90, 91). Very recently, β-lactams were demonstrated to induce expression of the *ccrC1* recombinase leading to SCC*mec* excision from the bacterial chromosome (92), an event upon which the element may be transferred to a conjugative plasmid (27). These findings point to our general lack of knowledge of how the expression of genes involved in transfer are regulated and moreover, how changes in gene expression affect the transfer processes. Surprisingly few studies have focused on globale expression of mobile genetic elements and how this is affected in different environments such as biofilm and during colonization.

CONCLUDING REMARKS

S. aureus is a very versatile human pathogen that readily adapts to changing environments and acquires antibiotic resistance genes through a number of different mechanisms. From epidemiological studies it has been apparent that there is extensive exchange of MGE particularly taking place in the hospital environment and recent experimental data have documented that the exchange of MGEs can occur within hours after contact. The mechanisms by which transfer occurs *in vivo* is still obscure but generalized transduction has been considered to be a main player. This notion may be supported by recent findings revealing the phenomenon of auto-transduction which suggest that phage and bacteria may even collaborate to acquire “useful” genes such as those providing antibiotic resistance. However conjugation appears to play a much greater role on transfer of ARGs than previously recognized. The ability of conjugative plasmids to mobilize smaller plasmids lacking most of the conjugative machinery and the transfer of plasmids lacking all known mobilizable elements but replicating as rolling circles suggest that the majority of staphylococcal plasmids may in fact be transferable by conjugation. Generally conjugation appears to be the preferred transfer mechanism when genetic material is travelling long genetic distances between species or even genera as also recently demonstrated by the evasion of lineage barriers by certain conjugative plasmids (76). On the other hand, recent studies suggest that transduction may also be highly promiscuous indicating that under the right conditions it may be equally important for long distance transfer. Most lacking in our information about transfer of ARGs is the knowledge of the conditions under which transfer

occurs. Studies from gut microbiota show that transduction (93) and conjugation (94) events are common in this environment but corresponding studies on transfer in the niche of colonizing *S. aureus* have only been addressed in few studies (33, 34). Research addressing these questions will not only provide us with valuable biological information about the basic processes underlying transfer of ARGs but will be crucial to limit the dissemination of antibiotic resistance in *S. aureus*.

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Acknowledgements

This work was supported by the Danish National Research Foundation's Centre of Excellence Bacterial Stress Response and Persistence, grant identifier DNRF120 (HI) and the Danish Research Council, Sapere Aude - Young Elite Researcher grant (JH).

651 Table 1: Antibiotic resistance genes carried on *S. aureus* mobile genetic
652 elements.

Type of Mobile Genetic Element	**Vector (example)**	**Antibiotic Resistance Gene**	**Antibiotic target**	**reference**
Plasmid	pC221, pC223, pUB112, pC194	*cat*	Chloramphenicol	(95)
	pUB110 (integrated conjugative), pSK41, pKKS825, pGO1	*aadD*	kanamycin, neomycin, paromomycin, tobramycin	(96)
	pMW2, pSAS, pI147, pI258	*blaZ*	Penicillin (b-lactam antibiotics)	(97, 98)
	pUB110, pSK41	*ble*	Bleomycin	(99)
	pSCFS1	*cfr*	Chloramphenicol, florfenicol, and clindamycin	(100)
	pSK1, pSK16	*dfrA*	Trimethoprim	(101)
	pI258	*ermB*	MLSB resistance (macrolides: erythromycin, lincosamides: clindamycin, streptogramin B)	(102)
	pE194, pUSA03	*ermC*	MLSB resistance (macrolides: erythromycin, lincosamides: clindamycin, streptogramin B)	(102)
	pUB101	*fusB*	Fusidic acid	(103)
	pMG1	*ileS-2*	Mupirocin	(104)
	pMS97	*mphBM*	Macrolide antibiotics	(105)
	pMS97	*msrA*	Macrolide antibiotics	(105)
	pS194	*str*	Streptomycin	(106)
	pT181, pUSA02	*tetK*	Tetracyclines	(107)
	pKKS825	*vgaC*	Streptogramin A, lincosamides, and pleuromutilins	(108)
	pKKS825	*tetL*	Tetracyclines	(108)
	pKKS825	*dfrK*	Trimethoprim	(108)
	pIP524	*vgb*	Streptogramins type B	(108)
	pSCFS1	*cfr*	Florfenicol and chloramphenicol	(100)
	pSCFS1	*spc*	spectinomycin	(109)
	pSCFS1	*isaB*	clindamycin	(109)
	pTZ2162	*fosD*	Fosfomycin	(110)
	pTZ2162	*qacB*	multidrux efflux	(110)

	SAP049A	*aadE*	aminoglycosides	(111)
	SAP049A	*aphA*	Neomycin and kanamycin	(111)
	pKH21	*linA*	Linezolid	(112)
	SAP082A	*mupA*	mupirocin	(112)
	SAP082A	*tcaA*	teicoplanin	(111)
Transposon	Tn*4001*	*aacA-aphD*	Gentamycin, kanamycin, tobramycin	(113)
	Tn*552*	*blaZ*	b-Lactam antibiotics	(114)
	Tn*554*	*ermA*	MLSB resistance (macrolides: erythromycin, lincosamides: clindamycin, streptogramin B)	(115)
	Tn*551*	*ermB*	MLSB resistance (macrolides: erythromycin, lincosamides: clindamycin, streptogramin B)	(116)
	Tn*558*	*fexA*	Florfenicol, chloramphenicol	(100)
	Tn*554*	*spc*	Spectinomycin	(115)
(Integrative conjugative element (ICE))	Tn*5801*	*tetM*	tetracycline	(117)
	Tn*1546*	*VanA*	Vancomycin	(118)
	Tn*4001*	*aac6'-aph2"*	aminoglycosides	(119)
Staphylococcal chromosome cassette (SCC), §	SCC476 or SCCfus	*fusC*	fusidic acid	(120)
	pseudoSCC*mec* II.5	*ant(4')*	Tobramycin	(121)
	SCC*mec* III and SCCHg	*tet(K)*	Tetracyclines	(122)
	SCCM1 and SCC*mec* IIE	*spc*	spectinomycin	(123)
	SCCM1 and SCC*mec* IIE	*erm(A)*	MLSB resistance (macrolides: erythromycin, lincosamides: clindamycin, streptogramin B)	(123)
Staphylcoccus aureus pathogenicity island (SaPI)	SaPIj50	*bla*	Penicillin (b-lactam antibiotics)	(124)

	SaPI122	mdr	multidrug exporter protein	(125)
	SaRIfusB	fusB	fusidic acid	(126)

§ = other than mecA, mecC, mecI, mecR1 and blaZ

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Figure legends

Figure 1. Conjugation and mobilization. Conjugative plasmids carry an origin of transfer, *oriT,* and encode a DNA relaxase (mob) as well as a coupling protein and products for formation of the mating pore (tra). Mobilizable plasmids may carry an *oriT* that is recognized by the relaxase of the conjugative plasmid (a); encode their own relaxase/*oriT* pair that uses the mating pore of a conjugative plasmid (b) or express a replicative relaxase (rep) that recognizes the replication origin (oriV) and is compatible with the conjugative-plasmid coupling protein (c) (Modified from (15).

Figure 2. Phage-mediated DNA transfer in *S. aureus*. By generalized transduction **(A)** transducing phages (red) infect susceptible donor bacteria (1) and upon lysis new phages are produced as well as rare transducing particles (green) containing bacterial DNA. Upon a new round of infection, the DNA of the transducing particle is delivered and established in a recipient bacterium, the transductant (2). By autotransduction **(B)** bacteria that carry a lysogenic, transducing phage release phages (B1) that upon propagation on phage susceptible cells form phages and transducing particles (B2). The lysogenic cells can effectively take up such transducing particles and in contrast to (A) these will survive in environments with high phage concentrations as the integrated phage makes them immune to phage killing (B3).

674 **Figure 1**

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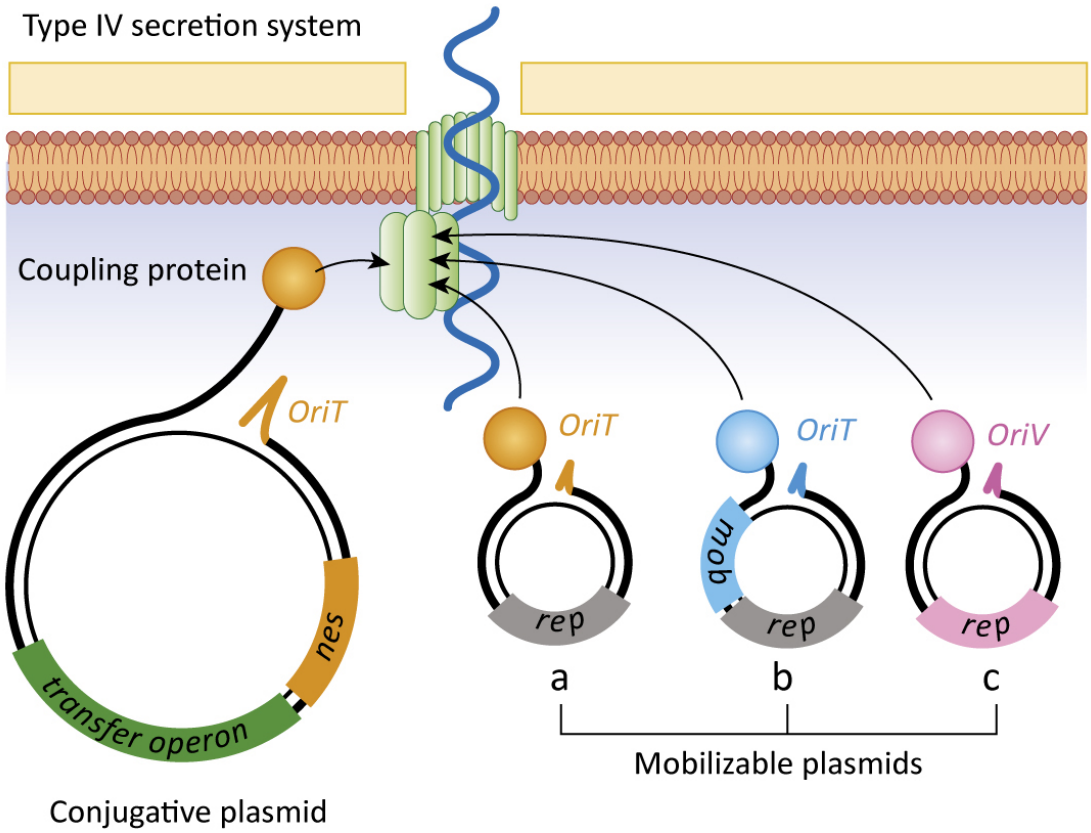

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679 **Figure 2**

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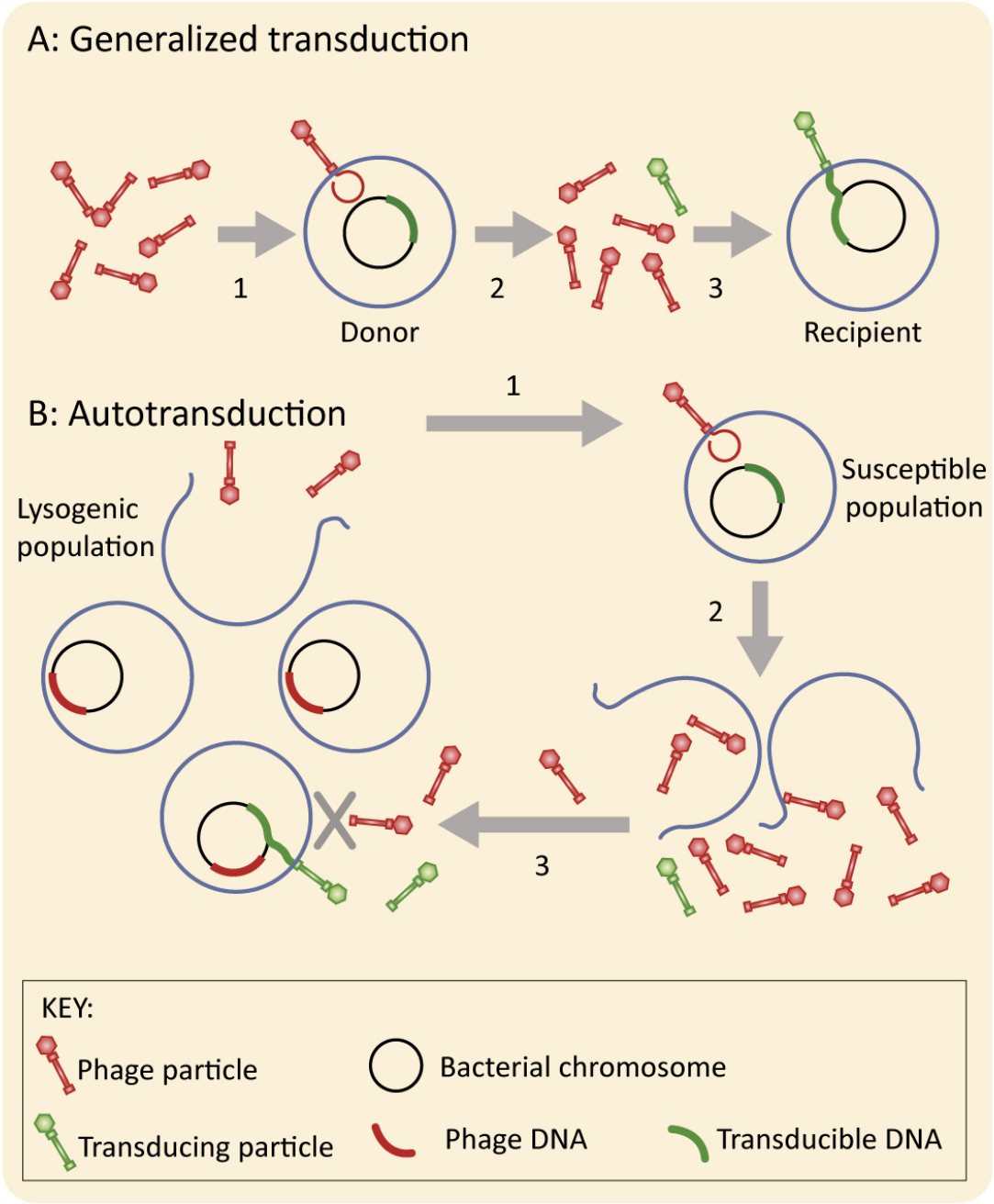

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